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Chromosomal imbalances in oral squamous cell carcinoma: Examination of 31 cell lines and review of the literature

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Summary Classical and molecular cytogenetic analysis, including fluorescence ***in situ*** hybridization (FISH) and chromosomal comparative genomic hybridization (CGH), were used to examine genetic changes involved in the development and/or progression of oral squamous cell carcinoma (OSCC). Of 31 OSCC cell lines studied, more than one-third expressed clonal structural abnormalities involving chromosomes 3, 7, 8, 9, and 11. Eleven OSCC cell lines were evaluated using CGH to identify novel genome-wide gains, losses, or amplifications. By CGH, more than half of the cell lines showed loss of 3p, gain of 3q, 8q, and 20q. Further, molecular cytogenetic analyses by FISH of primary tumors showed that the karyotypes of cell lines derived from those tumors correlated with specific gains and losses in the tumors from which they were derived. The most frequent nonrandom aberration identified by both karyotype and CGH analyses was amplification of chromosomal band 11q13 in the form of a homogeneously staining

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region. Our data suggest that loss of 9p and 11q13 amplification may be of prognostic benefit in the management of OSCC, which is consistent with the literature. The results of this study validate the relationship between these OSCC cell lines and the tumors from which they were derived. The results also emphasize the usefulness of these cell lines as ***in vitro*** experimental models and provide important genetic information on these OSCC cell lines that were recently reported in this journal.

Introduction

In the USA, more than 34,000 new cases of squamous cell carcinoma of the head and neck (SCCHN) are diagnosed annually.[1] If identified early, the prognosis of SCCHN is excellent. However, over the past several decades, SCCHN survival in Caucasians has only improved slightly and in African—Americans, the survival rate has actually decreased.[2] The overall 1, 5, and 10 year survival rates in the USA are 84%, 60%, and 48%, respectively.[1] Tobacco and alcohol are proven etiologic factors in SCCHN. They play a role in a process known as field cancerization, whereby all exposed oral epithelial tissue has the opportunity to become neoplastic.[3] Due to the field cancerization effect, patients with primary oropharyngeal SCCHN have a 20-fold increased risk of developing a second tumor in the same location.[4] Although a number of genetic markers have been associated with early diagnosis or prognosis of SCCHN, to the best of our knowledge, none have been implemented in routine clinical laboratory practice, although EGFR gene amplification is being considered, since it is being examined in lung cancer and EGFR inhibitors are available for therapy of amplified tumors.[5] Further, specific biomarkers for key genes responsible for SCCHN progression, such as EGFR, are essential for the intervention and treatment of SCCHN, but as yet only this gene has been translated to the clinic.

Classical and molecular cytogenetic analyses in conjunction with molecular genetic analyses by our group and others have revealed consistent genetic abnormalities associated with the development and/or progression of SCCHN. This research has revealed the specific genetic changes that take place during head and neck tumorigenesis, such as loss of chromosomal segments 3p, 5q, 7q, 8p, 9p, 11q, and 18q in addition to gains of regions of chromosomes 3q, 5p, 7p, 8q, and 11q.[6—11] This study had three goals. First, we karyotyped this series of SCCHN cell lines to identify the genetic alterations in each cell line. Second, we carried out FISH to confirm the relationship between cancer cell lines and the primary tumors from which they were derived. Third, we identified the common abnormalities in SCCHN and correlated our karyotype and CGH findings on the tumors and cell lines with clinical and pathological parameters to identify nonrandom genetic alterations that may serve as prognostic indicators in SCCHN.

Materials and methods

Tumor samples

After obtaining informed consent, biopsies of 31 oral squamous cell carcinomas (OSCC) were acquired from patients having surgical excision of their tumors at the University of Pittsburgh Medical Center (Table 1). Twenty-three of the tumors were primary OSCC, three patients' tumors were new primaries (UPCI:SCC026, UPCI:SCC068, and UPCI:SCC099), and five tumors were recurrences (UPCI:SCC002, UPCI:SCC080, UPCI:SCC084, UPCI:SCC090, UPCI:SCC104). Two patients with primary tumors included in this study also had new primaries or recurrences that were also included (UPCI:SCC016/UPCI:SCC026 and UPCI:SCC036/UPCI:SCC104). Five patients received radiation and/or chemotherapy treatment prior to tumor excision (UPCI:SCC002, UPCI:SCC016/UPCI:SCC026, UPCI:SCC078, UPCI:SCC090, UPCI:SCC104).

Upon receipt in the laboratory, the tumor specimen was subdivided into portions for cell culture, DNA extraction, and histopathologic diagnosis. Cell lines were established as described previously.[7,12] Three to four T-25 flasks of tumor cells from each established OSCC cell line were used for DNA extraction for CGH. Prior to DNA extraction, the tumor cells were rinsed in 1X HBSS, pelleted, and frozen at −80 °C.

Classical cytogenetic analysis

Cytogenetic harvests of the OSCC cell lines were carried out as described previously.[13] Metaphase spreads were trypsin-Giemsa banded and analyzed. Due to the extent of chromosomal instability in the tumor cell lines, composite karyotypes were not written, since they were very long. Instead, consensus karyotypes (cs), consisting of abnormalities observed in more than 40% of the cells, were prepared from at least eight cells per cell line. Karyotypes were written according to the ISCN (2005)[14] guidelines and images were captured and prepared using an AKS II Automated Karyotyping System (Imagenetics, now Vysis/Abbott Molecular Inc., Des Plaines, IL).

Fluorescence *in situ* hybridization (FISH)

To compare chromosome copy numbers between direct harvests of fresh tumor and adjacent oral mucosa cells from OSCC patients and cultured OSCC cells, we used dual-color FISH with alpha-satellite DNA probes for chromosomes 1, 3, 7, and 11, one labeled with biotin and the other labeled with digoxigenin. Alpha-satellite DNA probes for chromosomes 1 (D1Z5), 3 (D3Z1), 7 (D7Z1/D7Z2), and 11 (D11Z1), from Oncor, Inc. (Gaithersburg, MD), were utilized. At least 200 cells from each slide were counted and the number of nuclei with 0, 1, 2, 3, 4, and $\geqslant$5 signals was recorded. Results were compared between fresh and cultured tumor cells of the same case to assess whether karyotypic evolu-

Table 1 Clinical and histological data on patients/tumors examined in this study

Specimen (UPCI:)	Age/sex	S/A Hist.	Histological diagnosis	Site	Pathology stage	Stage	Tumor	Tx.	Outc.
SCC002	29/F	−/+	Well diff. SCC	Tongue	T4 N0	4	R	R	DOD
SCC003	65/F	+/+	Well diff. SCC	Tonsil	T1 N0	1	NP	−	NED
SCC016	82/F	−/+	Well diff. SCC	Tongue	T1 N0	1	NP	R	DOD
SCC026	82/F	−/+	Well diff. SCC	AR	T1 NX	1	R	R	DOD
SCC029B	84/M	+/+	Poorly diff. SCC	BM	T4 N2	4	NP	−	DOD
SCC030	38/M	+/+	Poorly diff. spindle cell SCC	FOM	T4 N2b	4	NP	−	DOD
SCC032	60/M	+/+	Mod. diff. SCC	RT	T2 N2b	4	NP	−	DOD
SCC036	56/M	+/+	Mod. diff. spindle cell SCC	Tonsil	T3 N1	3	NP	−	AOD
SCC040	50/M	−/+	Mod. diff. SCC	T	T2 N2	4	NP	−	DOD
SCC044	57/F	+/+	Mod. diff. SCC	BOT	T2 N2c	4	NP	−	DOD
SCC045	54/M	+/+	Mod. diff. SCC	RT	T2 N0	2	Prior P	−	NED
SCC051	62/M	+/+	Mod. diff. adenoid SCC	RT	T3 N2b	4	NP	−	DOD
SCC056	76/M	+/+	Mod. diff. SCC	T	T3 N2b	4	NP	−	AOD
SCC062	18/F	+/+	Well diff. SCC	T	T3 N0	3	NP	−	NED
SCC066	75/F	+/+	Well diff. SCC	AR	T1 N0	1	NP	−	NED
SCC068	60/M	+/+	Well diff. SCC	T	T3N1	3	NP	−	NED
SCC070	34/F	+/+	Mod. diff. SCC	RT	T3 N1	3	R	−	NED
SCC072	61/F	+/+	Mod. diff. SCC	Tonsil	T3 N2b	4	NP	−	NED
SCC074	51/F	−/−	Poorly diff. SCC	AR	T4 N1	4	NP	−	NED
SCC077	57/M	+/+	Mod. diff. SCC	FOM	T2 N2	4	NP	−	AOD
SCC078	60/M	−/+	Mod. diff. SCC	FOM	T2 N0	2	R	R/C	DOD
SCC080	66/M	+/+	Basaloid SCC	BOT	T1N0	−	R	R	DOC
SCC081	87/F	+/+	Mod. diff. SCC	AR	T4 N0	4	NP	−	DOD
SCC084	51/M	+/+	Mod. diff. SCC	RT	T2 N2b	4	R	−	DOD
SCC089	58/M	+/+	Mod. diff. SCC	Tonsil	T4 N2b	4	NP	−	DOD
SCC090	46/M	+/+	Poorly diff. SCC	BOT	T2 N0	2	R	R	NED
SCC099	52/M	+/+	Mod. diff. SCC	FOM	T1 N0	1	R	−	NED
SCC103	27/F	+/−	Well diff. SCC	T	T1 N0	1	NP	−	NED
SCC104	57/M	+/+	Mod. diff. SCC	FOM	T4 NX	4	R	R	AOD
SCC111	69/F	+/+	Poorly diff. SCC	FOM	T1 N1	3	NP	−	NED
SCC125	78/F	+/+	Mod. diff. SCC	AR	T4 N2b	4	NP	−	DOC

M, male; F, female; S, smoking; A, alcohol; Hist., history; −, negative; +, positive; Diff., differentiated; Mod., moderately; AR, alveolar ridge; BM, buccal mucosa; BOT, base of tongue; FOM, floor of mouth; RT, retromolar trigone; Grade, TNM Staging Classification as set forth by the American Joint Committee for Cancer; R, Recurrence; NP, new primary; Tx., previous treatment; R, radiation; C, chemotherapy; −, no previous treatment; Outc., outcome; DOD, dead of disease; NED, no evidence of disease; AOD, alive with disease; DOC, dead of other causes with as many as 11 years followup.

tion occurred in culture. In addition, comparisons were made between the classical and molecular cytogenetic results to evaluate the reliability of FISH in composing a molecular karyotype from interphase cells.

Comparative genomic hybridization (CGH)

Genomic DNA for CGH analysis was isolated from frozen tumor cell lines; DNA from normal male peripheral blood cells served as a control. CGH analysis was completed following the procedure described by du Manoir et al.[15] Slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and viewed under an Olympus fluorescence microscope equipped with a filter wheel and attached to a charged-couple device (CCD) camera interfaced with the Cytovision CGH software package (Applied Imaging, Santa Clara, CA). A 100× oil immersion objective was used for image acquisition.

The computer determined the ratio of the test DNA to the control DNA along the length of each chromosome by measuring the fluorescence intensities of the tumor and control DNA. At least five metaphases were captured for each OSCC cell line, resulting in 7–20 profiles of each chromosome. By averaging the individual ratio profile for each chromosome from multiple metaphases, the computer generated the overall ratio profile based on previously set guidelines[16] and plotted it beside the corresponding ideogram.

Results

Patient characteristics are shown in Table 1 and consensus karyotypes of the OSCC cell lines in Table 2. OSCC cell lines revealed high frequencies of aneuploidy with multiple chromosome rearrangements; ploidy levels ranged from diploid to pentaploid. Some tumors were comprised of a heterogeneous population of cells, although similar aberrations were observed between subpopulations. Structural rearrangements included unbalanced translocations, Robertsonian

Table 2 Consensus (cs) karyotypes from 29 OSCC cell lines; aberrations are present in more than 40% of the cells from that population

Specimen (UPCI:)	Passage # at harvest	Consensus karyotypes
SCC002	8	81−84⟨4N⟩,XXX,−X,del(1)(p33),der(1)t(11;1)(1;12)(q13;q25p11;p11),der(1)t(1;?;19)(q44;?;q11),−2,−3,−3,−4,der(4)t(4;14)(q11;q11),−5,del(5)(p11),−6,−6,der(6)t(6;10)(q11;q11)x2, −7,−8,del(8)(p21),del(9)(q21q31),−10,−10,−13,−14,der(14)t(6;14)(?q15;p12),−15,−16,−17,−18,−18,+19,+20,+20,−21,+22,+3mar5,+mar12,+mar14[cs8]
SCC003	2	38−42,X,−X,+2,−3,−4,−4,i(5)(p11),−8,−9,−10,−10,der(11)hsr(11)(q23)add(11)(q25),−12,−13,i(13)(q10),−14,−15,add(15)(p11),+16,−18,−20,−21,der(21)t(14;21)(q11;p11),−22, +mar2,+2mar4,+mar7[cs9]/76−79⟨3n⟩,XX,−X,+1,+2,−3,−4,+i(5)(p11),+7,−9,+der(11)hsr(11)(q23)add(11)(q25)x2,+i(13)(q10)x2,add(15)(p11),+18,+19,der(21)t(14;21)(q11;p11)x2, +mar2,+2mar4[cs3]/83⟨4N⟩,XX,−X,−X,−2,−3,−3,−4,−4,−4,−4,+i(5)(p11),−6,−8,−9,−9,−10,der(11)hsr(11)(q23)add(11)(q25)x2,−12,−12,−13,−13,i(13)(q10)x2,−14,−14,−14, −15,−15,−15,add(15)(p11),−17,−20,−21,−21,−21,der(21)t(14;21)(q11;p11),−22,−22,+2mar2,+4mar4,+mar7
SCC016	1	58−75,XX,−X,add(1)(p21),+del(1)(p22),+del(1)(q21),−2,−4,−5,−7,−7,−8,−9,i(9)(q11),der(10)t(7;10)(p11;p11),+hsr(11)(q13),−12,−13,−14,−14,−18,−18,−20,+mar1,+mar3, +mar4[cs13]/41−55,X,−X,+add(1)(p21),+del(1)(p22),+del(1)(q21),−2,+3,+i(3)(q10),+4,−7,−8,−8,i(9)(q11),−10,der(10)t(7;10)(p11;p11),+11,+hsr(11)(q13),−12,−13,−14,+16,+19,−20, −21,+22,+mar1,+mar4[cs4]
SCC026	2	41−50,X,−X,der(1)t(1;11)(p36.3;q13)hsr(11)(q13),+del(1)(q11),−2,del(3)(p24),+der(3)t(3;7)(p11;p11),del(4)(q21),del(9)(p13),−10,i(11)(q10),−12,−13,−13,−14,add(15)(p11),−18, −19,−21,add(21)(p11),−22[cs8]
SCC029B	2	75−80⟨3n⟩,XXY,del(1)(p22),+2,der(3)t(3;15)(p11;q11)x2,+i(3)(p10),+5,−6,der(7)t(7;14)(q11;q11),+der(7)t(7;14)(q11;q11),+der(7)t(3;7)(q11;q11),+der(7)t(6;7)(p11;q11),+8,−9, hsr(11)(q13q23),+hsr(11)(q13q23),+12,+13,−14,−14,−14,−15,del(15)(q24),i(16)(q10),+i(16)(q10),+17,−18,add(18)(q21),+19,+20,+i(20)(p10),−21,der(21)t(14;21)(q11;p11), −22[cs9]/81−84,XXY⟨4n⟩,−Y,del(1)(p22),−3,der(3)t(3;15)(p11;q11)x2,−4,+4,−5,−5,−6,der(7)t(6;7)(p11;q11.2)x2,+der(7)t(7;14)(q11;q11),+der(7)t(3;7)(q11;q11),−8,−8,−9, −9,−10,−10,hsr(11)(q13q23)x2,−12,−14,−14,−14,−15,del(15)(q24)x2,i(16)(q10)x2,+17,−18,add(18)(q21)x2,+i(20)(p10),−21,−21,der(21)t(14;21)(q11;p11),−22[cs7]
SCC030	1	60−72,X,−X,−Y,+del(1)(p22),−2,+3[4],−3[4],der(3)t(3;4)(q11;q12),−5,der(6)t(3;6)(q11.2;q11),+7,−8,+9,+10,del(11)(q21),+del(11)(q21),−12,−13,−14,−14,−15,add(15)(p11), −16,der(16)t(5;16)(q14;p13.3),−17,−17,−18,−18,−19,−21,−22,+mar1,+mar2[cs10]
SCC032	1	38−44,XY,−2,del(3)(p21),−4,−5,der(5)t(5;?)(q31;?),der(7)t(7;14)(q11;q11),−8,+del(9)(p13),+i(9)(q10),−10,der(11)(pter → q25::?::hsr::q13 → qter),−14,−14,der(15)t(15;21)(p11;q11), −16,−18,der(19)t(19;?)(q13.4;?),−22[cs9]
SCC036	2	57−64⟨3N⟩,X,−X,Y,add(1)(p31),del(1)(q24),+del(3)(p21),−4,−6,i(7)(p10),−8,−9,−10,−11,der(11)(pter → q23::q12 → q23::q12 → q23::q12 → q23),−12,−13,−13,−14,i(14)(q10),−15, add(15)(p11),−16,+17,−18,−18,−19,−20,−21,add(21)(p11),−22,−22[cs8]
SCC040	2	63−78⟨3n⟩,X,−X,−Y,−1,del(1)(p22)x2,+del(1)(q11),+i(1)(q10),−2,der(3)t(3;10)(p11;q11),+der(3)t(3;10)(p11;q11),+der(4)t(4;5)(q25;q22),i(5)(p10),+7,+del(9)(p21)x2,−10,del(10)(q11), del(11)(q13)hsr(11)(q13),−13,−13,−14,−15,−15,−17,−18,−20,−20,−21,−21,−21,−22,+mar1,+mar2,+2mar3,+mar4,+mar5[cs8]
SCC044	5	71−80⟨3n⟩,XX,−X,+2,del(3)(p21),−4[5],+5,−7,−8,i(9)(q11),+i(9)(q11),del(10)(p11),+11,+del(11)(q14)hsr(11)(q13)x2,+12,−13,−13,−13,der(14)t(14;21)(p11;q11),−15, der(15)t(15;22)(p11;q11),−16[5],−17,−18,+19,−20,−21,add(21)(p11)x2,−22[10],−22[5],+2mar1,+mar2,+mar3,+mar4,+mar5[cs11]/81−84⟨4n⟩,X,−X,−X,−X,del(1)(p21),+del(1)(q11), del(3)(p21)x2,+del(3)(p21),−4,−6,−7,−7,−8,−8,−9,i(9)(q11),−10,−10,del(10)(p11),del(11)(q14)hsr(11)(q13x2),+12,−13,−13,−13,−13,der(14)t(14;21)(p11;q11)x2,−15,−15,−15, der(15)t(15;22)(p11;q11),−17,−18,−18,−19,−20,−21,−21,−21,add(21)(p11),−22,−22,−22,+2mar1,+2mar2,+2mar3,+mar4,+mar5[cs3]
SCC045	4	64−81⟨3N⟩,X,−X,−Y,−2,−3,del(3)(p21),del(4)(p12),der(4)t(4;?)(q26;?),−6,i(7)(p10),der(8)t(8;14)(p11;q11),+i(8)(q10),−9,−10,+add(11)(q25),−13,der(14)t(14;21)(p11;q11),−15, −16,−16,+17,+19,+22,+mar1,+mar3[cs8]
SCC051	1	53−72⟨3N⟩,X,−X,−Y,del(1)(q25),+2,der(3)t(1;3)(q11;p11)x2,der(3)t(3;?)(p11;?),+der(3)t(3;?)(p11;?),+4,−5,der(5)t(5;6)(p11;p11),−6,−7,−7,−7,−8,−10,del(11)(q14)hsr(11)(q13), +del(11)(q14)hsr(11)(q13),−13,−13,−13,der(14)t(14;21)(p11;q11),−15,+16,−17,+19,−21,−21,−22,−22,+2mar1[cs10]
SCC056	9	33−40⟨2N⟩,X,+X,−Y,+2,−3,−5,del(6)(q16),−7,−8,−8,−9,i(9)(q10),der(11)(pter → 11q13::hsr::?),−12,−13,−14,−14,−15,−18,−21,−21,−22,−22[cs17]
SCC062	51	75−87⟨4N⟩,X,−X,−X,i(X)(q11),del(1)(p21)x3,del(1)(q25),+del(1)(q25),−3,del(3)(p13),−4,−4,i(5)(p10),−6,−7,del(7)(q21),−8,−9,−10,hsr(11)(q13),−13,−14,−14,−15, −15,i(15)(q10),−16,−18,−18,+20,+20,+21,+21,+22,+22[cs8]

SCC066	31	80−89⟨4N⟩,XX,−X,−X,−3,add(4)(q35),del(4)(q13),−7,−7,der(7)t(7;?)(q11;?),−8,−8,+9,−10,−10,−10,del(10)(p11),+11,−12,−13,−13,−14,−14,+15,+17,+18,−20,−20,−22[cs9]
SCC070	3	76−81⟨3N⟩,XXX,+2,+4,+5,+7,i(8)(q10),+i(8)(q10),+i(9)(q10)x2,der(11)hsr(11)(q13)add(11)(q25),+der(11)hsr(11)(q13)add(11)(q25),−13,−14,−14,+15,−18,−19,+22[cs5]/41−47, XX,−3,−5,−8,+i(9)(q10),der(11)hsr(11)(q13)add(11)(q25),−13,−14,−14[cs4]
SCC072	9	57−63⟨3N⟩,X,−X,−X,+del(1)(q11),+der(1)t(1;?)(p11;?),+2,i(3)(q10),−4,+5,+del(5)(p11),del(6)(q21),der(7)t(7;13)(q11;q11),+der(7)t(7;?)(q11;?),−14,der(14)t(14;22)(p11;q11)x2, −15,−15,−16,−18,−18,−19,−19,−21,−21,−21,−22,+mar1[cs7]/33−54⟨2N⟩,X,−X,del(1)(q11),+der(1)t(1;?)(p11;?),i(3)(q10),−4,+del(5)(p11),+del(6)(q21),der(7)t(7;13)(q11;q11), +der(7)t(7;?)(q11;?),i(8)(q10),−9,−10,add(11)(p14),+del(11)(q14),−13,−13,−14,der(14)t(14;22)(p11;q11),−15,−15,−18,−19,−21,−22,+mar1[cs4]
SCC074	2	37−47,X,−X,+3,−7,−8,+del(10)(q22),hsr(11)(q13),hsr(11)(q13),−13,−13,−14,−14,−15,−15,−18,+20[cs15]
SCC077	10	59−70,XXY,del(1)(p22),+del(1)(q21),−3,−3,−4,−5,−6,−6,−7,−7,−8,−10,der(11)t(11;13)(p11;q11),+der(11)t(11;15)(p11;q11),−12,−12,−13,−13,−14,−14,−15,−15, −16,add(19)(q13.4),+add(19)(q13.4),+20,+20,i(21)(q10),−22,−22,−22,+mar1[cs9]
SCC078	8	63−75,XXY,del(1)(p32),+2,+2,+del(3)(p21)x3,del(4)(q22),−5,−8,i(8)(q10),+9,−10,del(10)(q22),der(11)t(3;11)(q13;q25)hsr(11)(q23)x2,−12,−13,der(14)t(14;21)(p11;q11),−15, −17,−17,+18,−19,−21,−21[cs17]
SCC081	2	44−51,X,−X,+i(7)(p10),−8,+10,+15,−16,+20[cs17]
SCC084	1	40−45,XY,del(1)(q11),+i(1)(q10),+inv(2)(p16q24),−3,del(3)(p25),−4,−4,i(5)(p10),del(7)(q22),del(9)(p21),der(11)t(3;11)(q11;p11)hsr(11)(q13),−13,−18,−19,−21[cs20]
SCC089	4	65−80⟨3n⟩,XX,−X,+del(1)(p34.1),del(3)(p21),+del(3)(p21),del(4)(p14),+del(4)(p14),+6,+del(7)(q32)x2,−8,+9[8],9[7],−10,−11,hsr(11)(q13)x2,der(13)t(8;13)(q11;p11)x2, +der(13)t(8;13)(q11;p11),add(14)(p11),+i(14)(q10),+20,+20[cs19]
SCC090	5	58−89⟨3N⟩,XX,−Y,+1,+del(1)(q11),+2,der(3)t(3;?)(p11;?),+der(3)t(3;?)(p11;?),−4,+5,−8,+9,−10,−10,−11,der(11)t(11;15)(p11;q11),der(13)t(13;22)(p11;q11),−14, der(14)t(14;21)(p11;q11),der(14)t(14;22)(p11;q11),der(15)t(15;22)(p11;q11),−17,+18,+19,+20,−22,+mar1[cs19]
SCC099	5	39−45,XY,−9,i(9)(q10),−19,−22,der(22)t(9;22)(p11;p11)[cs17]
SCC103	10	103−129⟨5N⟩,XX,−X,−X,−X,+1,+2,del(3)(p13),−4,−5,i(5)(p10)x2,+7,+del(7)(q22)x2,−8,−8,i(8)(q10)x2,−9,−9,i(9)(q10),+10,+del(10)(q23)x2,+11, +der(11)(pter → q13::hsr::q14 → q25::hsr::?)x2,−13,+14,+der(14)t(11;14)(q11;p11)x2,−15,−16,−16,−16,−17,del(17)(p11)x2,−18,+20,+20,+20,−21,−22,−22,−22,+2mar1, +2mar2,1−3dmin[cs11]/58−63,X,−X,−X,−2,−3,−4,i(5)(p10)x2,−6,+del(7)(q22),−8,−8,i(8)(q10),−9,i(9)(q10),+10,+del(10)(q23),der(11)(pter → q13::hsr::q14 → q25::hsr::?), −13,+der(14)t(11;14)(q11;p11),−15,−16,der(16)t(16;17)(q24;q21),−17,del(17)(p11),−18,+20,+20,−21,−22,−22,+mar1,1−2dmin[cs3]
SCC104	5	60−74,XX,−Y,−1,+2,+der(3)t(3;?)(q11;?),−5,−6,−7,add(7)(q36),−8,−8,der(11)t(9;11)(q13;q22),−13,−13,der(14)t(11;14)(q11;p11),der(14)t(14;21)(p11;q11), −15,−15,i(15)(q10),+16,−17,+19,+add(19)(q13.4)x2,+20,+20,+20,−21,−21,−22,−22,+mar1,+mar2,+mar3[cs9]
SCC111	14	57−76⟨3N⟩,XX,−X,del(1)(q11),+del(3)(p14),+del(6)(q13),+der(7)t(1;7)(q11;q11),−8,i(8)(q10),+9,−11,−12,−13,−15,−15,−16,−18,−19,−21,−22,−22,+mar1,+mar2[cs19]
SCC125	6	42−63⟨3N⟩,X,−X,−X,der(1)t(1;2)(p11;p11),+2,−3,−6,der(8)t(8;13)(p11;q11),del(10)(p12),der(10)t(10;13)(p11;q11),−11,−13,−13,−13,der(14)t(14;22)(p11;q11), i(14)(q10),add(15)(p11),−17,−18,−18,−19,−20,−21[cs20]

translocations, deletions, isochromosomes, and homogeneously staining regions (hsrs). For illustration, a karyotype of UPCI:SCC103 is shown (Fig. 1).

Two patients in this study, UPCI:SCC016 and UPCI:SCC036, each had subsequent tumors that were karyotyped and analyzed. The patient with tumor UPCI:SCC016,

Figure 1 Representative karyotype from UPCI:SCC103 passage 10: 63⟨3n⟩,−X,−X,−Y,+der(2)t(2;?)(q10;?),−3,−4,−5,i(5)(p10),+del(7)(q22),−8,−8,i(8)(q10),+10,+del(10)(q23),+der(11)(pter → q13::hsr::q14 → q25::hsr::?),−13,+der(14)t(11;14)(q11;p11),−15,−16,der(16)t(16;17) (q24;q21),−17,del(17)(p11.1),−18,+20,−21,−21,−22,−22,+mar1,+mar2,+mar,+dmin.

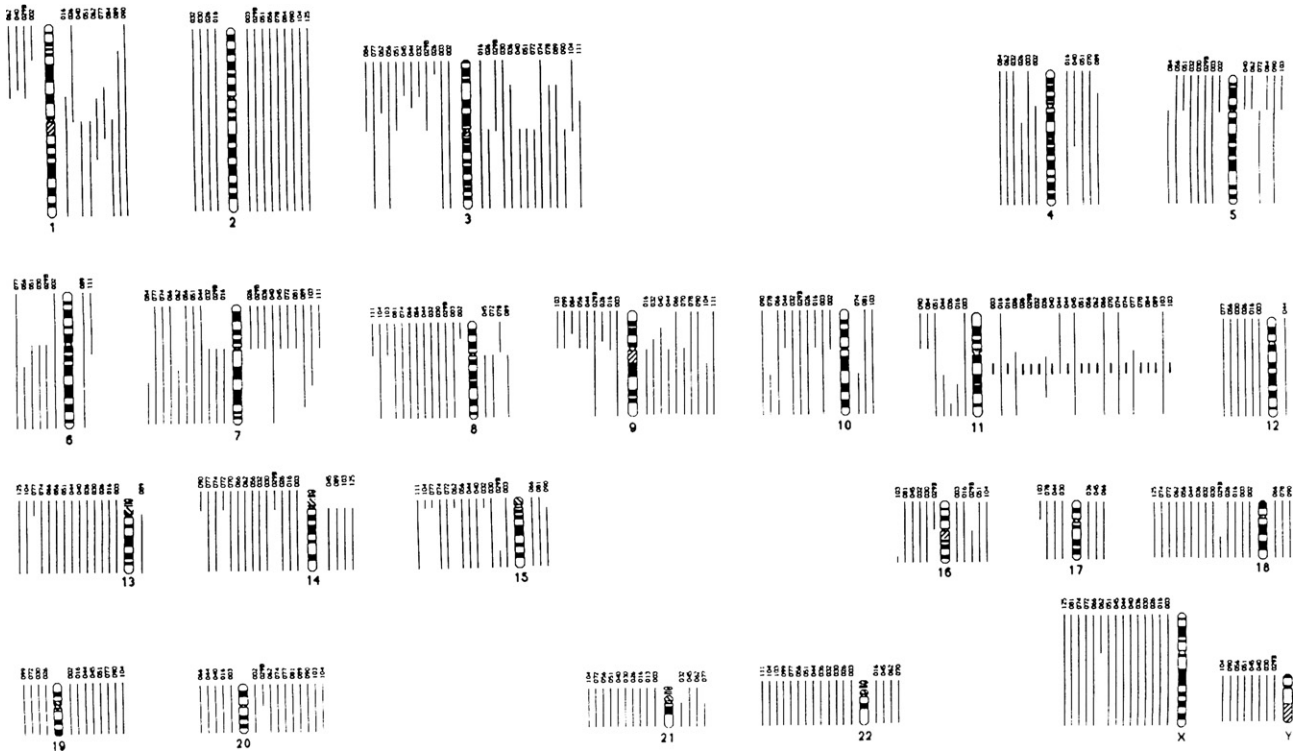

Figure 2 Summary of nonrandom chromosomal losses and gains identified by classical cytogenetic analysis of 30 OSCC cell lines. The cell line number corresponding to each line is marked above the lines. Lines to the left or right of the ideograms indicate regions of loss and gain, respectively. Thick lines denote gene amplification.

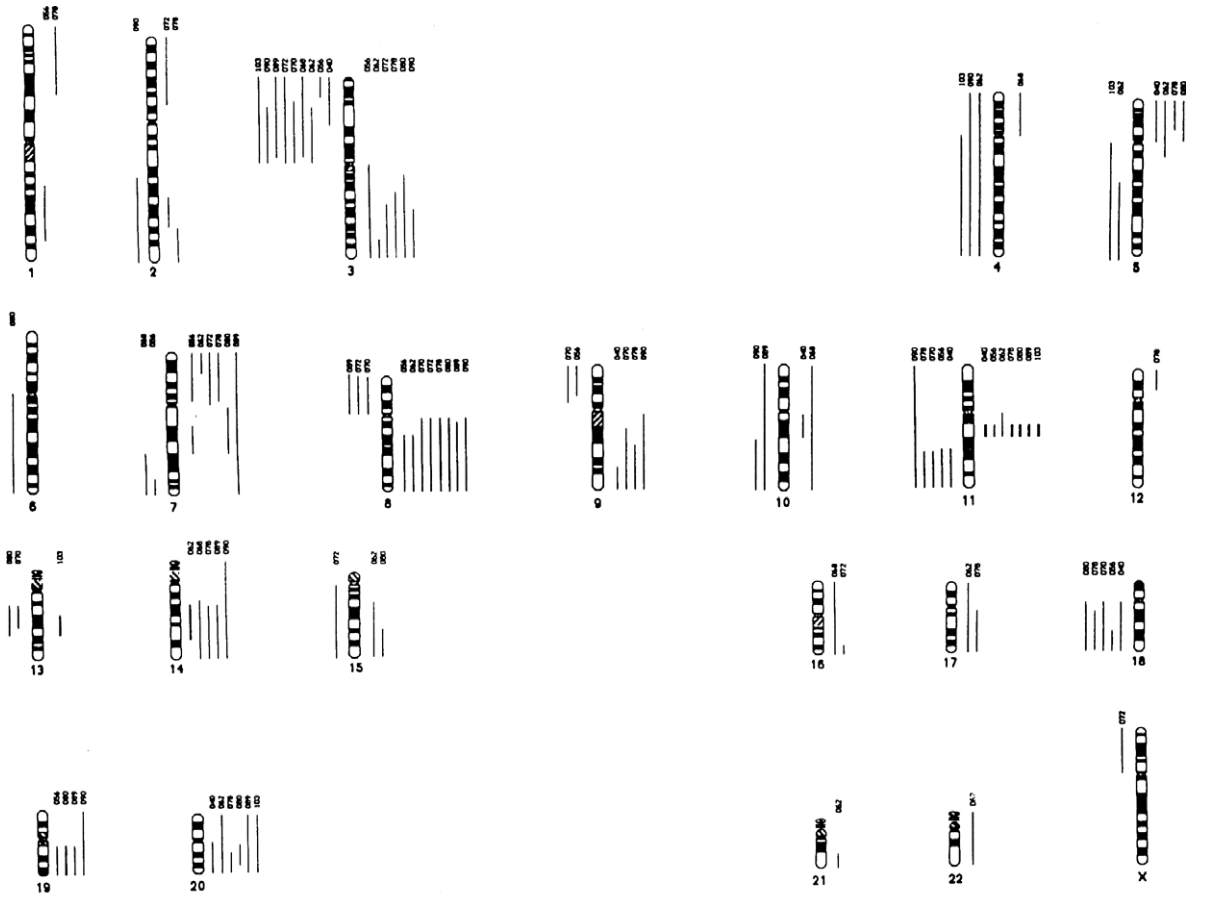

Figure 3 Summary of nonrandom chromosomal losses and gains identified by chromosomal CGH in 11 OSCC cell lines. The cell line number corresponding to each line is marked above the lines. Lines to the left or right of the ideograms indicate regions of loss and gain, respectively. Thick lines denote gene amplification.

a near-triploid tumor, had what was considered clinically a new primary tumor, UPCI:SCC026, which was near-diploid. Aside from amplification of chromosomal band 11q13, comparison of consensus karyotypes did not reveal consistent chromosomal rearrangements. Amplification of 11q13 was observed as an hsr in its native band in UPCI:SCC016 and as an hsr on a derivative chromosome 1 in UPCI:SCC026. The patient with tumor UPCI:SCC036 had what was considered clinically a recurrence, tumor UPCI:SCC104. Although both tumors were near-diploid, no similar chromosomal aberrations were observed. These findings suggest that the development of secondary primary tumors may have occurred independently from the initial tumors in both cases.

Summaries of chromosomal gains and losses by karyotyping and CGH analysis are illustrated in Figures 2 and 3, respectively. Tables 3 and 4 show losses and gains of chromosomal segments observed in at least 20% of 29 OSCC cell lines by karyotype analysis and 11 OSCC cell lines by CGH analysis, respectively. As seen in Table 3, karyotype analysis revealed that nonrandom loss of chromosomal segments occurred more frequently than chromosomal gains in this series of OSCC cell lines. However, CGH analysis showed that gains of genomic material occurred more frequently (Table 4). This observation can be explained by the ability of CGH to characterize gains and losses imparted by marker chromosomes of unknown origin in the consensus karyotypes (Table 2).

Comparison of classical cytogenetic and CGH analyses confirmed many chromosomal alterations. Deletions involving regions of chromosome arms 3p, 4q, 8p and chromosome 18 were found in at least 20% of the cell lines using both methods, as were gains involving chromosome arms 3q, 7p, 9q and chromosomes 19 and 20. Amplification of 11q13 was the most frequently observed abnormality by both karyotype and CGH analyses. However, CGH revealed three novel regions of amplification, 13q21 $\rightarrow$ q22, 14q21 $\rightarrow$ q24, and 19q13.1 $\rightarrow$ qter, each observed in one cell line. In addition, extrachromosomal representations of gene amplification (double minutes) were observed in UPCI:SCC103, and may have resulted from sequence amplification of the 13q21—q22 region.

We compared copy number for chromosomes 1, 3, 7, and 11 between 12 freshly harvested tumors (UPCI:SCC018, 020, 021, 023, 026, 029, 030, 044, 070, 074, 084, 089), corresponding adjacent mucosa cells from three of these (UPCI:SCC029, 030, 044), and the cell lines derived from 11 tumors (UPCI:SCC002, 003, 016, 026, 029, 030, 044, 070, 074, 084, 089). The results from the FISH analysis of chromosomes 1, 3, 7, and 11 in the OSCC are provided in

Table 3 Chromosomal losses and gains observed most frequently by classical cytogenetic analysis in 29 OSCC cell lines

Frequency of cell lines with loss/gain	Loss	Gain
⩾50%	Y	11q13 amplification
40—49%	3pter → p24 8pter → p21 13 14pter → p11 15pter → p11 18 22 X	3q11 → qter
30—39%	3pter → p14 3pter → p21 7q22 → qter 8 9pter → p21 10pter → p11 14 21	2 7pter → p11 9q13 → qter 20
20—29%	3pter → p11 4q21 → qter 5pter → p11 6q16 → qter 12	1q21 → q25 19

Table 4 Chromosomal losses and gains observed most frequently in 11 OSCC cell lines by chromosomal comparative genomic hybridization[a]

Frequency of cell lines with loss/gain	Loss	Gain
⩾50%	3pter → p24 3p21 → p12	3q27 → qter 8q11 → qter 8q13 → qter 20q12 → q13.2
40—49%	11q22 → qter 18q22 → qter	3q24 → qter 7pter → p21 11q13 amplification
30—39%	18q12 → qter	5pter → p14 9q32 → qter 14q21 → qter
20—29%	4q11 → qter 8pter → p11 18q11 → qter	7q21 → q22 19q13.1 → qter

[a] Cell lines (UPCI:SCC at passage, p): 040p7, 056p7, 062p24, 068p10, 070p4, 072p10, 078p5, 080p17, 089p5, 090p4, 103p4.

Supplementary Table 1 and Supplementary Figures 1—4. Sixteen tumors were examined for aneuploidy of chromosomes 1 and 3 using centromeric DNA probes. For chromosome 1, 14 (87.5%) tumors were aneuploid, 10 tumors showed gain of chromosome 1, and four tumors showed loss. For chromosome 3, 15 (94%) tumors were aneuploid, 11 tumors showed only copy number gain of chromosome 3, while four tumors showed both loss and gain of chromosome 3. Simultaneous loss and gain of chromosome 3 is best explained by the presence of more than one population of tumor cells identified using FISH, which enables analysis of a larger number of cells than classical cytogenetic analysis. Eight tumors (67%) were aneuploid for chromosome 7, all of which expressed copy number gain. For chromosome 11, 15 tumors were examined for copy number gain or loss. Eleven tumors (73%) were aneuploid for chromosome 11, all of which showed gain of chromosome 11. From these data, it appears that in OSCC, chromosomal gain is a much more frequent event than chromosomal loss. However, many of the chromosomal ''gains'' most likely represent tumor cell populations that have become tetraploid from aborted cytokinesis in a diploid progenitor population. Therefore, these observations may not represent true aneuploidy, but rather, the evolution from a diploid to a tetraploid tumor. Supplementary Figures 1, 2, 3, and 4 are histograms that display data collected using FISH with alpha-satellite probes to chromosomes 1, 3, 7, and 11, respectively. Each histogram contains data from those specimens for which fresh and cultured tumor cells were available: UPCI:SCC026, 029, 030, 044, 070, 074, 084, and 089. For comparison, a peripheral blood cell control that was hybridized with the same probe is shown in each graph. Overall, the results illustrated in the histograms demonstrate increased aneuploidies of chromosomes 1, 3, 7, and 11, providing further evidence for their nonrandom involvement in OSCC tumorigenesis. For the most part, the number of signals observed for each chromosome in the direct harvest of the tumor cells corresponded to that of the cultured tumor cells from the same case. Therefore, since corresponding numerical alterations were observed in the directly harvested tumor cells and the cell cultures, these data suggest that little karyotypic evolution has occurred in culture. Clearly, the clone with two copies of the chromosome tested was often reduced in the cell lines, suggesting that our efforts to remove diploid fibroblast-like (stromal) cells from the OSCC cultures was successful, thus validating the successful culturing of OSCC tumor cells. Our results suggest that cultured cells reflect the chromosomal status of the tumors from which they were derived and that analysis of cultured cells is informative of features of the tumors. In addition, the observation of the same number of signals in the direct and cultured tumor cells suggests that clonal selection of the tumor cells has not occurred *in vitro*.

Discussion

In this study, classical and molecular cytogenetic analyses were used to survey gross genome-wide changes occurring in OSCC cell lines. Our FISH studies of primary tumors showed that the karyotypes of cell lines derived from those tumors correlated with specific gains and losses in the tumors from which they were derived, validating the use of cell lines in OSCC research. Worsham et al.[17] reported similar findings from their study of two head and neck squa-

mous cell carcinomas. Using multi-probe FISH, they compared the ploidy levels observed in cultured tumor cells with those of tumors from which they were derived by examining histologic sections. Cytogenetic aneuploidy was confirmed for the chromosomes examined, demonstrating that the genetic aberrations identified in the tumor cells after a prolonged period in culture were consistent with the genetic aberrations in the original tumors.

Classical cytogenetic analysis was carried out using both short- and long-term cultured tumor cell harvests from 29 OSCC cell lines (Table 2). The majority of breakpoints involved in the chromosomal rearrangements were located in the centromeric regions, consistent with earlier reports of aberrations in head and neck tumors.[6,18]

As shown in Table 3, karyotype analysis revealed that nonrandom loss of chromosomal segments occurred more frequently than gains in this series of OSCC cell lines, whereas CGH analysis showed an increased proportion of chromosomal gains rather than losses (Table 4). This observation may be explained by the ability of CGH to further characterize previously unidentified marker chromosomes (Table 2). Overall, analysis of these OSCC lines revealed chromosomal losses at 3p, 4p, 8p, 11q, and i8q and gains at 3q, 5p, 7p/q, 8q, 9q, 11q, 14q, 19q, and 20q.

Regions of chromosome loss

Chromosomal regions lost in OSCC cells most likely harbor tumor suppressor genes that contribute to tumorigenesis. Multiple discrete regions of loss were observed on the short arm of chromosome 3: 3pter → p24, 3pter → p21, 3pter → p14, 3pter → p11 and 3p21 → 12, consistent with cytogenetic studies of head and neck cancer showing loss of 3p as a key genomic change.[6,9,19] Loss of 3p has been implicated as an early event in SCCHN, as its absence has been shown in dysplastic oral lesions.[20–22] Previously, we too demonstrated alterations or loss of at least one *FHIT* allele in 22 of 26 OSCC cell lines,[23] consistent with the findings that changes in the *FHIT* gene and/or protein expression play a key role in the development and/or progression of SCCHN.[24,25] In addition, loss of heterozygosity has been reported for 3pter → p24, 3p21.3, 3p14 → cen, 3p21.3 → 3p13, 3p23 → 3p21.3, 3p25, 3p24.2 → p22, 3p21.1 → p12, and 3p14.1 → cen.[26–28] Taken together, these studies suggest the presence of at least three tumor suppressor genes located on the short arm of chromosome 3 that may play a role in head and neck tumorigenesis. Several candidate tumor suppressor genes, including *HRCA1*, *RAR*β, and *FHIT*, reside in regions of chromosome 3p that are most commonly deleted.[29–31] Our laboratory has been examining alterations in *FANCD2*, which codes for a critical DNA damage response protein at 3p26.3, also noted to map to a hotspot of genetic alterations by Weber et al.[32]

In this study, loss of chromosome 9p, including breakpoints at 9p21, 9p13, and 9p11, and gain of chromosome 9 from 9q13 → qter and 9q32 → qter were observed. Deletions of the 9p region have been frequently reported in head and neck cancers by both classical cytogenetic and molecular analyses.[9] Previous studies identified 9p allelic loss in preinvasive lesions, suggesting that its absence is an early event in SCCHN development.[22,33,34] Band 9p21 is known to contain several tumor suppressor genes, *p15/CDKN2B/MTS2*, *p16/CDKN2A/MTS1*, *p18/CDKN2C*, and *p19/CDKN2D*, which encode cyclin-dependent kinase inhibitors.[35] Alterations in any or all of these genes may lead to uncontrolled cell proliferation through loss of cell cycle checkpoint control, resulting in tumorigenesis.[9]

Interestingly, 95% (19/20) of patients whose tumors contained an intact 9pter—p21 region did not develop a new primary tumor in the followup period, compared to 50% (4/8) of patients with 9p loss, who developed new primary tumors. This observation suggests that deletions in tumor suppressor genes located on chromosome 9p, such as *p15/CDKN2B* or *p16/CDKN2A*, may also be important for the development of subsequent SCCHN lesions. This is consistent with the findings of Rosin et al.[22] which indicate that 3p and 9p loss in premalignant oral lesions at former cancer sites have a 26.3-fold increase in risk of developing a second oral malignancy compared with lesions that retained these two chromosomal regions.

CGH analysis of our OSCC cells revealed both loss of chromosomal material from 11q22 and amplification of chromosomal band 11q13 in close to 50% of cases examined. Loss of distal 11q will be discussed in the context of 11q13 amplification below.

Nonrandom chromosomal loss of chromosome 18q has frequently been reported in SCCHN.[8,18,36–38] In the current study, deletion of the entire long arm of chromosome 18 was observed most frequently by classical cytogenetic analysis, whereas three distinct breakpoints (18q11, 18q12, and 18q22) were identified using CGH. The region 18q21 contains tumor suppressor genes including *DCC*, *SMAD2*, *SMAD4*, *SERPINB4*, *SERPINB5*, and *SERPINB13*.[39–44] LOH of the *DCC* gene has been observed more frequently in patients with decreased SCCHN survival than in those who remain disease-free.[45] In addition, normal expression of the *SERPINB13* gene has been found in oral keratinocytes, oral mucosa, and skin, but exists in lower levels in OSCC, suggesting that its absence may allow SCCHN progression.[46]

Regions of chromosome gain

Many studies have demonstrated gain of chromosomal material on 3q through the formation of an isochromosome 3q, although the majority of extra copies observed in our cell lines resulted from unbalanced translocations. Gains of 3q[8,36,37,47] may suggest the presence of a cancer-related gene, such as the ataxia-telangiectasia and rad3-related gene (*ATR*) at band 3q22—q24. Our recent studies and those of others have shown that overexpression of *ATR* could result in tumor initiation and/or progression by promoting chromosomal instability through an aberrant DNA damage response.[48–50] More telomeric to *ATR* lies the *AIS* (*TP73L*) gene, a human p53 homologue at 3q26—q27, which is present in extra copies, although not amplified technically ($\geqslant$2.5 copies on a diploid background or $\geqslant$5 copies on a tetraploid background), and overexpressed in squamous cell carcinomas.[51,52] *AIS/TP73L* has been shown to transform rat 1a cells into a malignant phenotype, suggesting that it may also be important for SCCHN tumorigenesis.[51]

Gain of chromosome 7p, previously reported by both classical cytogenetic analyses and CGH,[9] was also observed

in this series of OSCC cell lines. Merritt et al.[53] first showed amplification and overexpression of the epidermal growth factor gene, mapped to 7p12.3—p12.1, in SCCHN. Subsequent studies by Grandis et al.[54] have demonstrated that increased protein expression of EGFR and its ligand, TGF-α, are significant predictors for disease recurrence and decreased overall survival, which suggests that EGFR may be a key oncogene for a subset of SCCHN tumors. More recently, it has been shown that the targeted EGFR inhibitor, Erlotinib (Tarceva®) prolonged survival in patients with advanced non-small cell lung cancer who had progressed after standard chemotherapy.[5]

Chromosomal 14q gains in our OSCC cell lines are consistent with the findings in primary tumors from Speicher et al.[8] and Järvinen et al.,[55] despite recent findings that showed loss of 14q to be more common.[19] Therefore, the significance of 14q copy number gain in OSCC cell lines remains unclear, although the same genes on 14q that are amplified may be involved in driving the chromosomal gain (see below).

Despite our CGH finding that gain of chromosomal material from 20q was present in more than 50% of OSCC cell lines analyzed, 20q amplification has not been detected in primary SCCHN tumors and thus, the prognostic significance in OSCC remains unknown.[8,19] However, amplification of 20q in node-negative breast cancer cells has been shown to result in decreased disease-free patient survival.[56] Key genes that are gained or amplified on 20q12—13 in breast and/or pancreatic cancer include *ZNF217*, *NCOA3*, *AIB1*, *MYBL2*, *CTSZ*, *AURKA*.[57—59]

Gene amplification

Our CGH analyses revealed three novel regions of amplification, 13q21 → q22, 14q21 → q24, and 19q13.1 → qter, each observed in one cell line. Amplification of 13q21 was reported in one case of OSCC.[60] The amplicon involving 14q12 → q22.1 has been reported in prostate cancer, including overexpression of the *TITF1* and *MAP4K5* genes.[61] The *TITF1* gene codes for thyroid transcription factor 1, a sensitive and specific marker of pulmonary adenocarcinoma, but not extrapulmonary adenocarcinoma. The protein is highly expressed in lung adenocarcinoma, strongly expressed in small cell lung carcinoma and thyroid tumors, although not expressed in SCC of the lung. The *MAP4K5* gene codes for mitogen activated protein kinase kinase kinase kinase 5, which maps to 14q11.2—q21 and is a member of the serine/threonine protein kinase family that activates the Jun kinase in mammalian cells. The chromosome 19 amplicon was reported in a comprehensive, correlated study of copy number and gene expression with pathway analysis in laryngeal SCC by Järvinen et al.[55] and by Nessling et al.[59] to involve the *CCNE1* and *AKT2* genes in breast cancer.

CGH analysis of our OSCC cells revealed both loss of chromosomal material from 11q22 and amplification of chromosomal band 11q13 in close to 50% of cases examined. Loss of chromosome material from 11q has been suggested to occur as an intermediate stage in tumor progression, following dysplasia but preceding carcinoma *in situ*.[20] First reported by Jin et al.,[62] the region distal to 11q13 has been thought to contain at least two tumor suppressor genes implicated in SCCHN progression and a wide variety of other solid tumors.[63] Loss of distal 11q was reported in laryngeal SCCs by Järvinen et al.[55] Haploinsufficiency, as a result of loss of copies of genes involved in the cellular response to DNA double strand breaks including *ATM*, *MRE11A*, *CHEK1*, and *H2AFX*, has been shown to promote chromosomal instability through gene mutation, amplification, and structural chromosome aberrations.[49,64—66] Our group has shown that OSCC cell lines with distal 11q loss are characterized by a deficient DNA damage response and loss of sensitivity to ionizing radiation.[50] Further, we have shown in OSCC cell lines that 11q13 amplification usually occurs as a result of breakage—fusion—bridge cycles initiated by a break at the common chromosomal fragile site, FRA11F,[13,67,68] consistent with other studies in the literature which have demonstrated that gene amplification may result from breakage at chromosomal fragile sites.[69,70] Common fragile sites are thought to lead to difficulties during DNA replication, and when under replication stress as a result of carcinogen exposure or conditions that occur in precancerous lesions or cancer cells, may lead to replication fork stall or collapse, cell cycle checkpoint induction, and DNA repair.[71] In OSCC, defects in the DNA damage response as a result of genetic loss distal to the 11q13 amplicon and double strand breakage at FRA11F as a result of replication fork collapse may prompt aberrant DNA repair to occur through sister chromatid fusion, leading to chromosomal instability through breakage—fusion—bridge cycles.[13,67,68] Alternatively, Gibcus et al.[72] propose that these 11q13 fragile sites are not involved in the breakage necessary for 11q13 amplification, but that the presence of both syntenic transitions and segmental duplications determine the pattern of amplification per the model proposed by Narayanan et al.[73]

Gene amplification of 11q13 as an hsr has been observed in more than 30—50% of SCCHN tumors[7,11,60,62,72,74—76] as well as tumors of the aerodigestive tract, bladder, breast, pancreas, lung, ovary, and liver.[77] The Hittelman group found 11q13 amplification to be an early change in SCCHN,[78] although more recent studies have shown that gain of the 11q13 region along with loss of 11q14 → qter are associated with metastatic lesions of HNSCC,[19] suggesting that 11q13 amplification may be a late event in HNSCC progression. Thus, the timing of this event is controversial. We have observed two copies of the chromosome bearing the 11q13 hsr in many of our near-triploid and near-tetraploid subpopulations, suggesting that the amplification occurred prior to tetraploidization. Chromosomal band 11q13 contains the cyclin D1 gene (*CCND1*)[35] and other genes such as *EMS1*,[79—81] *FGF3* (*INT2*),[7,82] *FGF4*(*HSTF1*),[83] and the tumor amplified and overexpressed sequence genes 1 and 2 (*TAOS1*/*ORAOV1*, *TAOS2*/*TMEM16A*).[84,85] Since the RNA transcript and CCND1 protein have been overexpressed in OSCC cells, it is thought to play an important role in SSCHN progression.[86] In addition, rapid disease recurrence and poor survival in cases with lymph node involvement have been shown to correlate with cyclin D1 protein overexpression.[86—89] Our more recent results, confirmed by those of Gibcus et al.[72] have shown that all but four of 13 genes in the 11q13 amplicon core are overexpressed and this expression appears to be coordinated.[85] We proposed that 11q13 amplification may be driven by a cassette of genes that provide growth or metastatic advantage to cancer cells. This is supported by the

finding that the human 11q13 amplicon core is syntenic to mouse chromosomal band 7F5, which is frequently amplified in chemically induced murine OSCC. Freier et al.[90] proposed that coamplification of *EMS1* and *SHANK2* might have a cooperative effect on OSCC tumor cell motility and invasiveness. Thus, genetic alterations involving chromosome 11 appear to play a key role in OSCC.

Analysis of our data revealed that amplification of 11q13, identified by the presence of an hsr, appeared to be associated with tumor site. Amplification of 11q13 was found to occur more frequently in tumors of the tongue, retromolar trigone, and buccal mucosa. Of the 11 OSCC cell lines we examined by CGH, the most significant finding was the correlation of 11q13 amplification with decreased patient survival. All patients whose tumors lacked 11q13 amplification (6/11) survived, compared to only one of five patients whose tumors expressed 11q13 amplification. This observation further validates the use of 11q13 amplification as a biomarker for patient prognosis, as has previously been reported by several groups.[88,91–93]

Although previous investigations suggest that *CCND1* is the primary cause for OSCC progression in 45% of cases, there are several genes remaining within the 11q13 amplicon that are simultaneously amplified and in some cases, overexpressed at a higher frequency.[85,86] Due to the identification of genome-wide nonrandom genetic events in OSCC by classical cytogenetic, CGH, and now cDNA microarrays, targeted studies assessing the significance of amplification and overexpression of other genes within the 11q13 amplicon may allow for the elucidation of other key genes associated with HNSCC progression. Further investigation of the biochemical pathways impacted by 11q13 amplification and distal 11q loss are in progress.

In spite of the presence of chromosomal instability, the karyotypes of the cell lines appear to be quite stable over time. Reshmi et al.[94] analyzed UPCI:SCC040 by G-banding and spectral karyotyping at passage 16 and two 'clones' derived by single cell cloning were karyotyped at passage 35. Despite the observation that individual OSCC tumor cells express unique karyotypes and at times unique numerical and structural aberrations, many abnormalities within and among malignant cell populations appear to be clonal and remain constant over time in spite of chromosomal instability resulting from chromosome segregation defects.[95] This suggests that clonal abnormalities provide a selective growth advantage for high grade tumors, consistent with the suggestion by Albertson et al.[96] Our cell lines have been examined in our laboratory as late as passage 68 and appear to be remarkably stable. However, any cell line used as a laboratory tool should be tested regularly by DNA fingerprinting and/or karyotyping to be certain that it is the same cell line as expected and has not evolved or more likely, been replaced by HeLa cells or another cell line, including one from another species. Further, cell lines should also be examined at the earliest passage available. Thus, our series of SCCHN cell lines[12] and those of other investigators[97] may serve as useful laboratory tools for basic and translational research studies. Similar to our recent finding of loss of radiosensitivity associated with distal 11q loss,[50] or the previous finding of *EGFR* gene amplification, further investigation of clonal chromosomal abnormalities and genes located therein may lead to useful biomarkers for determining which patients will benefit from targeted therapies and should ultimately assist in the treatment of SCCHN.

Conflict of Interest Statement

None of the authors have any financial or personal relationships with other people or organizations that could inappropriately influence or bias their contribution to this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.oraloncology.2007.05.003.

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